Chemical Derivatization of Hydroxyatrazine for Gas Chromatographic Analysis

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The reaction conditions for silylation, methylation, and alkylation of hydroxyatrazine [2-hydroxy-4-(ethylamino)-6-(isopropylamino)-s-triazine] were examined and the resultant derivatives were analyzed by gas chromatography using an alkali flame ionization detector (AFID). The silylated derivatives lowered the detector sensitivity considerably. Methylated or alkylated derivatives of hydroxyatrazine can be readily gas chromatographed. A method was developed for the simultaneous detection of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] and hydroxyatrazine at residue levels in soil by gas chromatography. The procedure is based on the extraction of sample with methanol and separation of atrazine and hydroxyatrazine in the extract by column chromatography on acidic alumina. This is followed by derivatization of the separated compounds, column clean-up on silica gel, and subsequent determination by gas chromatography. The lower limit of sensitivity for both atrazine and hydroxyatrazine by this method is approximately 0.5 ppm. The alkylation reaction developed can also serve as a useful chemical confirmatory test for s-triazines and their hydroxy analogs in biological systems.

Replacement of the chloro group by a hydroxyl group is one of the degradative reactions of 2-chloro-s-triazine herbicides in soils (Knuesli et al., 1969; Goswami and Green, 1973). The 2-hydroxy analogs, which are the main metabolites, cannot be readily gas chromatographed and it is necessary to derivatize them in order that they may be quantitated. Schroeder et al. (1972) used a chlorination reaction for converting hydroxycyprazine to cyprazine which was subsequently determined by gas chromatography. Montgomery et al. (1969) treated hydroxysimazine obtained from corn plants with diazomethane in an attempt to prepare the methoxy derivative. However, they found no detectable ^{14}C residues in the gas chromatographic effluent following injection of diazomethane-treated [^{14}C]hydroxysimazine, even though methoxytriazines are volatile and easily determined chromatographically. The application of the silylation technique to the gas chromatographic analysis of hydroxytriazines and metabolites has been attempted by several workers (Bakke and Price, 1973; Montgomery et al., 1969; Flint and Aue, 1970). Treatment of hydroxysimazine and metabolites with hexamethyldisilazine and trichlorosilane produced silyl derivatives which were readily analyzed by gas chromatography (Montgomery et al., 1969). Reaction of hydroxy derivatives of simazine, atrazine, and propazine with bis(trimethylsilyl)trifluoroacetamide (BSTFA) in closed vials at 150° for 15 min gave single gas chromatographic peaks and small amounts of side products (Flint and Aue, 1970). Spiked soil and corn samples showed a minimum detectable level of hydroxyatrazine of not better than 1 ppm.

The aim of this investigation was to compare the gas chromatographic response of methylated, silylated, and alkylated reaction products, using hydroxyatrazine as a model compound. Furthermore, methylation with diazomethane or alkylation with NaH-alkyl halide of atrazine and hydroxyatrazine from spiked soil samples was also attempted for their simultaneous determination by gas chromatography.

EXPERIMENTAL SECTION

Chemicals. All solvents were pesticide grade and used as received. All reagents were analytical grade. Analytically pure samples of atrazine [2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine] and atratone [2-(ethylamino)-4-(isopropylamino)-6-methoxy-s-triazine] were obtained from CIBA-Geigy. Hydroxyatrazine (2-hydroxy analog) was prepared by acid hydrolysis of atrazine according to Gysin and Knuesli (1960).

Derivatization. (i) *Silylation.* A solution (1 ml) containing atrazine (34 μg) in methanol was transferred into a 15-ml screw cap centrifuge tube, the solvent was removed in a stream of dry air, and about 0.25 ml of BSTFA was added. The tube was tightly closed and the mixture was heated in an oil bath at 150° for 30 min. The tube was removed from the bath, cooled to room temperature, and extracted with hexane (10 ml × 3). The hexane extract was concentrated to 1 ml in a stream of dry air and analyzed by gas chromatography.

(ii). *Methylation.* A solution (1 ml) containing hydroxyatrazine (2–121 μg) in methanol was transferred into a 15-ml screw cap centrifuge tube and an excess (100–200 mg) of ethereal diazomethane solution (prepared from Diazald, Aldrich Co., Inc., Milwaukee, Wis.) was added until the yellow color persisted. The tube was tightly closed and the content was allowed to stand at room temperature for 3 hr with occasional shaking. The solvent was then evaporated to dryness in a stream of dry air. The residue was dissolved in 1 ml of hexane and analyzed by gas chromatography, using atratone as a reference standard.

(iii) *Alkylation.* A solution (1 ml) containing atrazine (50 μg) or hydroxyatrazine (5–99 μg) in dimethyl sulfoxide (Me_2SO) was transferred into a 15-ml screw cap centrifuge tube and then 1 ml of hexane, about 20 mg of NaH (prewashed with hexane), and 0.5 ml of CH_3I were added. The tube was tightly closed, heated in an oil bath for 1 hr at 70°, and cooled to room temperature, and the excess NaH was decomposed with water. The mixture was then extracted with hexane (10 ml × 3). The hexane layers were collected in a graduated centrifuge tube, concentrated in a stream of dry air to 1 ml, and analyzed by gas chromatography by using alkylated hydroxyatrazine as a reference standard. The latter was prepared by carrying out the alkylation reaction on a macro scale and purifying the product on a silica gel column (1.4 cm diameter, 7.5 g, activated, prewashed with hexane). The column was first eluted with 180 ml of 10% diethyl ether in hexane and then with 180 ml of 30% diethyl ether in hexane. The first eluate was discarded and the second eluate was evaporated to dryness and weighed. The residue was dissolved in hexane and used as a reference standard.

Effect of Time on the Yield of Derivatized Material. Silylation (68 μg of atrazine, 30 μg of hydroxyatrazine),

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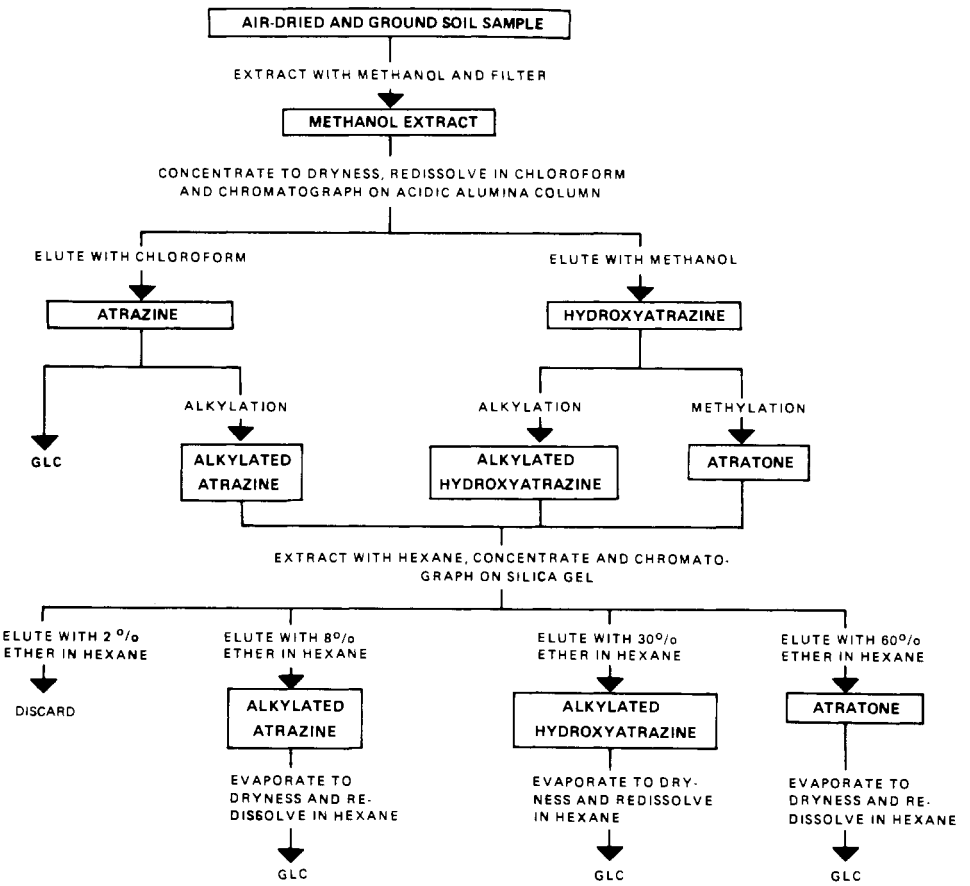

Figure 1. Schematic diagram for the analysis of atrazine and hydroxyatrazine residues in soil.

methylation (30 μg of hydroxyatrazine), and alkylation (68 μg of atrazine, 30 μg of hydroxyatrazine) reactions at 150°, room temperature, and 70°, respectively, were carried out for 0.25, 0.5, 1, 2, 4, or 16 hr. The product was isolated as above and analyzed by gas chromatography.

Effect of Temperature on the Yield of Derivatized Material. Silylation (210 μg of atrazine or hydroxyatrazine) and alkylation (68 μg of atrazine, 30 μg of hydroxyatrazine) reactions for 30 and 60 min, respectively, were carried out at temperatures ranging from 100 to 200° and from 25 to 100°, respectively. The derivatized material was analyzed by gas chromatography.

Determination of Residues in Soil Fortified with Atrazine and Hydroxyatrazine. The air-dried and ground soil (5 g) sample was fortified with a mixture of atrazine and hydroxyatrazine at 0.5- and 1-ppm levels. The solvent was allowed to evaporate and the sample was mixed thoroughly. The sample was extracted with 100 ml of methanol in a mechanical shaker for 2 hr and then filtered under suction. The sample residue was washed with methanol (100 ml) and the combined filtrate was evaporated to dryness on a rotary evaporator. The dried residue was redissolved in several 5–10-ml portions of chloroform and introduced into an acidic alumina column (2.4 cm diameter, 20 g of aluminum oxide, Woelm, acidic, activity 1, prewashed with chloroform). The column was first eluted with 150 ml of chloroform and then with 150 ml of methanol. The two eluates which contained atrazine and hydroxyatrazine, respectively, were evaporated to dryness on a rotary evaporator. A portion of the dried compound from the first eluate (chloroform eluate) was dissolved in hexane and an aliquot of this solution was injected into the gas chromatograph for atrazine analysis. The compounds obtained in the two eluates described above were methylated or alkylated (Figure 1). The derivatized compounds were dissolved in hexane and transferred to a silica gel column (1.4 cm diameter, 3 g, activated, prewashed with hexane) topped with 0.5 cm of anhydrous sodium sulfate. Interfering co-extractants were eluted with 60 ml of 2% diethyl ether in hexane. Alkylated atrazine, alkylated hydroxyatrazine, or methylated hydroxyatrazine (atratone) was eluted from the column with 60 ml of 8, 30, or 60% diethyl ether in hexane, respectively. The eluates were evaporated to dryness in a stream of dry air, then the derivatized residues were redissolved in hexane and analyzed by gas chromatography. Figure 1 shows a schematic diagram of the method described above.

Gas Chromatography. The gas chromatograph was a Pye series 104, Model 124 fitted with an alkali flame ionization detector (AFID) having a CsBr Annulus. Two columns, 5 ft × 0.25 in. o.d. glass tubes packed with 3% SE-30 or 3% Carbowax 20M coated on 80–100 mesh Chromosorb WHP were used. The operating conditions were: column temperature, 190° (SE-30) or 200° (Carbowax 20M); detector and injector temperatures, 200 and 190°, respectively; carrier gas, nitrogen flow rate 40 (SE-30) or 50 ml/min (Carbowax 20M).

Mass Spectrometry. For the mass spectrum, an aliquot of the solution containing approximately 1–3 μg of the derivatized material was injected into a Pye gas chromatograph, Model 104, interfaced to a Du Pont mass spectrometer Model 490 with a jet separator.

NMR Spectrometry. The proton NMR spectrum was obtained on a Varian A-60 spectrometer. The sample was dissolved in spectrophotometric grade deuterated chloroform-d_1.

RESULTS AND DISCUSSION

Silylation. Reaction of hydroxyatrazine with BSTFA led to a mixture of mono-, di-, and trisilyl derivatives, the proportion of which varied with the time and temperature of the reaction (Figure 2). Silylation of hydroxyatrazine at

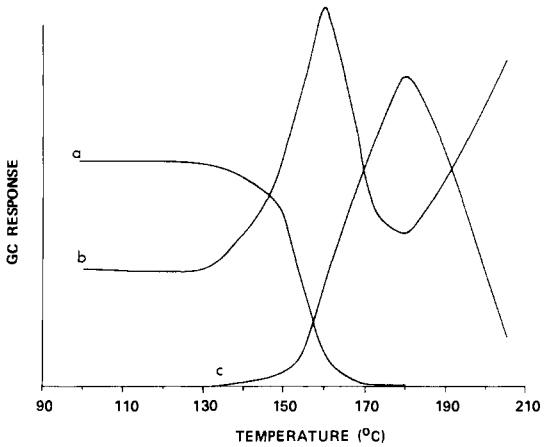

Figure 2. Effect of temperature on silylation of hydroxyatrazine (a) monosilyl; (b) disilyl; and (c) trisilyl derivatives.

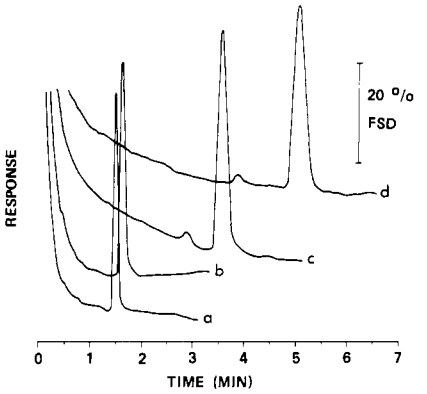

Figure 3. Gas chromatograms of (a) alkylated hydroxyatrazine; (b) alkylated atrazine; (c) atratone; (d) atrazine. GC conditions: glass column, 5 ft × 0.25 in. o.d. packed with 3% Carbowax 20M on Chromosorb WHP; column, detector, and injector temperatures, 200, 200, and 190°, respectively; carrier gas (nitrogen) flow rate, 50 ml/min; chart speed, 1 in./min; attenuation, 1×10^2.

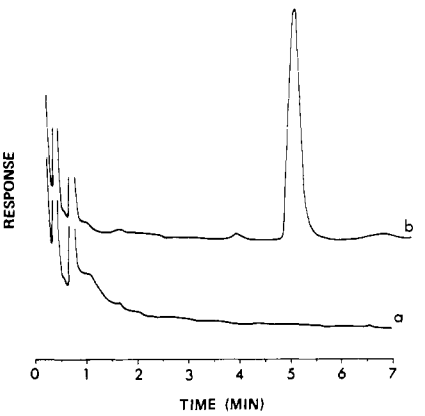

Figure 4. Gas chromatograms of extracts from blank and fortified (atrazine and hydroxyatrazine, 1-ppm levels) soils. GC conditions same as in Figure 3: (a) blank soil; (b) fortified soil.

150° for 30 min gave 43.5, 41.3, and 2% yields of mono-, di-, and trisilyl derivatives, respectively. The yields of the silylated derivatives were calculated by comparing peak areas with that of atrazine. It was assumed that the AFID response to N was the same for both atrazine and silylated derivatives. The relative retention times for mono-, di-, and trisilyl derivatives were 1.32, 1.65, and 2.45, respectively, with respect to atrazine (atrazine retention time, 1.3 min, SE-30 column). The monosilyl derivative had a molecular ion ($M\cdot^+$) at m/e 269 with the intense fragment ion at m/e 254 ($M\cdot^+ - CH_3$) and a fragment ion at m/e 196 [$M\cdot^+ - Si(CH_3)_3$]. Similarly, the di- and trisilyl derivatives had molecular ions at m/e 341 and 413, and fragment ions at m/e 326, 368 and 398, 340, respectively. The yield of the monosilyl derivative decreased above 130° and at 160° mainly the disilyl compound was obtained (Figure 2). At higher temperatures the trisilyl derivative was obtained as the main reaction product. Silylation of hydroxyatrazine for 16 hr at 150° produced no mono- but relatively higher amounts of the di- than the trisilyl derivative.

Atrazine itself could be derivatized by this technique but it required a high reaction temperature and gave a single product having the relative retention time of 1.4 with respect to atrazine. At lower temperatures (<120°) no reaction took place, but at 190° derivation of atrazine was obtained in over 90% yield. The latter was found to be the monosilyl derivative and had a molecular ion peak at m/e 289. Comparison of its proton NMR spectrum with that of atrazine showed two overlapping quartet resonances centered at τ 6.5 attributed to the CH_2 protons of the *N*-ethyl group coupling with the CH_3, and the NH proton had changed to only one quartet at τ 6.45. This indicated that silylation had occurred exclusively at the *N*-ethyl position. Presumably steric effects hindered reaction at the *N*-isopropyl position. Reaction time appeared to have no effect on the yield of silylated atrazine.

Although silylation technique is adequate for confirmatory test, in general, it is not recommended for use with an AFID. The SiO_2, formed by combustion, deposits on salt tip and probe, and may also block the flame jet (Coward and Smith, 1971). This would result in temporary poisoning of the salt surface and loss of sensitivity. It was observed that repeated injections of the silylated material invariably led to a gradual loss of sensitivity. For example, ten consecutive injections of silylated hydroxyatrazine resulted in a loss of sensitivity of the AFID by about 40%. For these reasons the silylation technique was not used further in this study.

Methylation. Montgomery et al. (1969) observed that diazomethane did not methylate hydroxysimazine. However, Cochrane and Purkayastha (1973) contended that some methylation of an OH group substituted on a *s*-triazine nucleus should be possible although not quantitative. They pointed out that a possible complication in such a reaction is the lactam–amide tautomerization of the -C(OH)=N moiety. In our experience, methylation of hydroxyatrazine with diazomethane is possible under the experimental conditions used in this study. Hydroxyatrazine was converted to atratone in 50–80% yield (average yields from 121, 51, and 2 μg of hydroxyatrazine were 74, 71, and 68%, respectively). The methylated derivative had a molecular ion peak at m/e 211 and a relative retention time of 0.74 with respect to atrazine (Figure 3, atrazine retention time 5 min, Carbowax 20M column). For the AFID detector, 6.9 ng of this compound gave a 50% full scale deflection. The diazomethane–ether solution used for esterification should be deep yellow before it is used for the reaction. A lighter color solution, which implies low concentration of diazomethane, results in incomplete reaction. A considerable variation in the yields of the methylated hydroxyatrazine was obtained when the esterification was carried out under the conditions described. Therefore, the methylation reaction cannot be regarded at present as quantitative. Similar yields of the methylated material were obtained when the esterification was carried out for longer periods (16 hr). However, several unknown major peaks were observed in the gas chromatograms possibly due to the slow polymerization of diazomethane molecules. In this work esterification for 3 hr at room temperature was considered adequate for the detection of hydroxyatrazine in soil extracts.

Table I. Recovery of Atrazine and Hydroxyatrazine from Fortified Soil Samples

Derivatization method	Atrazine, ppm			Hydroxyatrazine, ppm		
	Added	Recovered	% recovery	Added	Recovered	% recovery
No derivatization	0.5	0.443	88.6			
	1.0	0.945	94.5			
Methylation				0.5	0.357	71.4
				1.0	0.705	70.5
Alkylation	0.5	0.405	81.0	0.5	0.263	52.6
	1.0	0.900	90.0	1.0	0.600	60.0

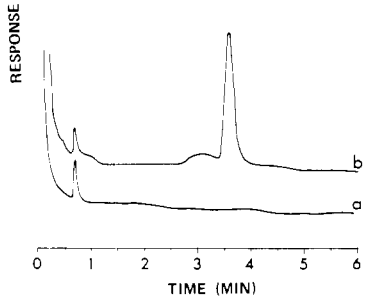

Figure 5. Gas chromatograms of methylated extracts from blank and fortified (atrazine and hyroxyatrazine, 1-ppm levels) soils (60% ether–hexane eluate from clean-up column). GC conditions same as in Figure 3: (a) blank soil; (b) fortified soil.

Alkylation. The use of Me_2SO was preferred because of greater solubility of hydroxyatrazine in this solvent. On a macro scale alkylation of 5.9 mg of hydroxyatrazine yielded a compound (eluted by 30% diethyl ether in hexane fraction on a silica gel column) weighing 4.6 mg (64.4% yield). The hydroxyatrazine derivative gave a single gas chromatographic peak and had a relative retention time of 0.31 with respect to atrazine on Carbowax 20M column (Figure 3). The alkylated derivative had a molecular ion peak at m/e 239 and its mass and NMR spectra indicated that the OH and both NH ethyl and NH isopropyl groups had been alkylated to give *N,N'*-dimethylatratone. Alkylation of atratone gave an identical product (*N,N'*-dimethyl derivative) in greater than 90% yield. Under the reaction conditions described 99, 25, and 5 μg of hydroxyatrazine gave average yields of alkylated product of 61, 60, and 64%, respectively. About 10 ng of this compound gave 50% full scale deflection. When the derivation was carried out at lower temperatures (<70°) or for shorter periods (<1 hr) reaction was not complete and resulted in low yields. On the other hand, alkylation at higher temperatures or for longer periods resulted in side products as evidenced by numerous gas chromatographic peaks and the yields of the alkylated product were also low. Alkylation of atrazine (50 μg) at 70° for 1 hr produced a derivative in about 90% yield. The compound had a molecular ion peak at m/e 243, with a strong fragment ion at m/e 228 ($M^{\cdot+} - CH_3$) corresponding to the loss of a methyl group from an isopropyl group. The material was shown to be *N,N'*-dimethylatrazine and had a relative retention time of 0.34 with respect to atrazine (Figure 3). For a 50% full scale deflection only a 3.6-ng sample was required. Reaction time or temperature appeared to have no effect on the optimum yield of the alkylated atrazine.

Recovery of Atrazine and Hydroxyatrazine from Fortified Soil Samples. The applicability of methylation and alkylation reactions described above has been tested for the confirmation and detection of atrazine and hydroxyatrazine at 0.5- and 1-ppm levels in soil. Figure 4 shows the gas chromatograms of the blank and fortified soil samples without any derivatization and clean-up. A few unknown peaks appeared in the chromatograms due to coextractives but they did not interfere with the atrazine peak. Recoveries of atrazine from the fortified soil samples at the 1.0- and 0.5-ppm levels ranged from 86 to 95% (Table I).

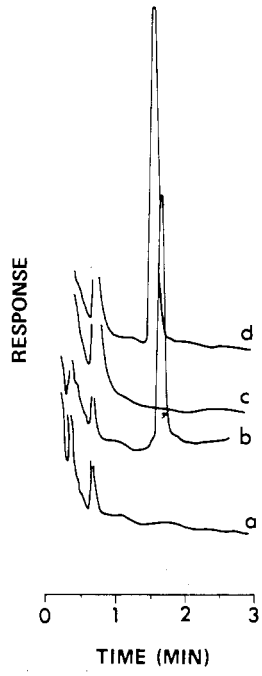

Figure 6. Gas chromatograms of alkylated extracts from blank and fortified (atrazine and hydroxyatrazine, 1-ppm levels) soils. GC conditions same as in Figure 3: (a) blank soil (8% ether–hexane eluate from clean-up column); (b) fortified soil (8% ether–hexane eluate); (c) blank soil (30% ether–hexane eluate); (d) fortified soil (30% ether–hexane eluate).

Clean-up of the derivatized soil extracts by silica gel column (Figure 1) was found adequate for removal of the most interfering peaks, as well as for the separation of derivatized products in different fractions. Preliminary experiments showed that about 90–95% of the derivatized reference standards were recovered in the eluting solutions (Figure 1). Figure 5 shows the chromatograms of methylated and cleaned-up soil extracts. Methylation of extracts from the fortified soil samples gave hydroxyatrazine recoveries (60% diethyl ether in hexane fraction, Figure 1) in the range of about 70% (Table I). The alkylation reaction produced very few extraneous peaks and much cleaner chromatograms were obtained after clean-up (Figure 6). Using the alkylation method the recoveries of atrazine (8% diethyl ether in hexane fraction, Figure 1) and hydroxyatrazine (30% diethyl ether in hexane fraction, Figure 1) from the fortified soil samples at the 1.0- and 0.5-ppm levels ranged from 81 to 90% and from 53 to 60%, respectively.

The data presented show that application of the meth-

ylation or the alkylation reaction constitutes a suitable method for confirmation and detection of hydroxyatrazine residue levels down to approximately 0.5 ppm when present in a soil sample. Although alkylated atrazine and alkylated hydroxyatrazine have overlapping retention times on a Carbowax 20M column (Figure 6), the former will not interfere with the gas chromatographic detection of the latter after column separation through silica gel (Figure 1). The derivation methods developed for hydroxyatrazine are believed to be applicable, with minor modification, to other 2-hydroxy-*s*-triazines.

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Adsorption of Benzimidazole Fungicides on Montmorillonite and Kaolinite Clay Surfaces

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The effect of pH on the adsorption by montmorillonite and kaolinite clays of three benzimidazole derivatives, thiabendazole (TBZ), 2-benzimidazole carbamic acid methyl ester (MBC), and benzimidazole, was investigated. Adsorption isotherms for TBZ and MBC on Ca and Na Wyoming bentonite showed that an increase in the acidity of the suspension resulted in a significant increase in the adsorption of the fungicide to the clay. The shape of the adsorption curves on Ca-bentonite at various pH values resembled simple titration curves with a midpoint 2.5–3.5 pH units above the pK_a of the respective fungicides. The midpoint for TBZ on Na-kaolinite was in the vicinity of its pK_a. The fungicide MBC did not adsorb on kaolinite clay even at pH 2. These findings suggested adsorption on mineral clay surfaces by protonation of these basic organic molecules. Protonation and adsorption were not directly related to the basicity of the molecule. An increase in $CaCl_2$ concentration resulted in a decrease in the adsorption of the fungicides. Both fungicides were adsorbed in aqueous solutions on MgO and not adsorbed on Al_2O_3.

Benzimidazole derivatives were introduced as systemic fungicides capable of controlling a wide spectrum of plant diseases (Bollen and Fuchs, 1970; Edgington et al., 1971). Application of benomyl or thiabendazole (TBZ) as a soil drench for the control of vascular wilt diseases was found to require extremely large dosages which for many crops are not economical (Erwin, 1973). This was presumably due to tight adsorption of these fungicides to soil and to the immobility of these chemicals in the soil (Baude et al., 1974). The rate of degradation of 2-benzimidazole carbamic acid methyl ester (MBC) and TBZ in the soil was shown to be relatively slow, with a half-life for MBC of several months (Baude et al., 1974).

The mechanism by which these chemicals are bound and/or degraded in the soil is not clear. A study was therefore conducted to examine the mechanism of interaction of benzimidazole derivatives with the soil clay minerals. It has been shown (Mortland, 1970; Bailey and White, 1970) that the acidic environment of clay surfaces provides conditions for protonation of organic bases. Absorption of triazine herbicides or purines and pyrimidines on clay surfaces takes place due to protonation of these molecules (Brown and White, 1969; Weber, 1970b; Yamane and Green, 1972; Lailach and Brindley, 1969).

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The effectiveness of soil drenches with benzimidazole fungicides, i.e., the movement and persistence of these chemicals in the soil, may be dependent on their interactions with clay surfaces.

EXPERIMENTAL SECTION

Materials. *Chemicals.* Analytical standards of TBZ and MBC were obtained from E. Merck and from E. I. DuPont de Nemours & Co. Inc., as were the fungicides recrystallized from technical TBZ and MBC.

Analytical benzimidazole was purchased from BDH Ltd.; magnesium oxide, for chromatography, from BDH Ltd.; aluminum oxide, neutral, activity I, from E. Merck.

Clays. Montmorillonite No. 25 API, Project No. 49 (bentonite), was from Upton Wyoming, and a commercial supply of bentonite was obtained from Upton Wyoming. During this study no real differences were found between the two sources of bentonite and montmorillonite. Ca- and Na-montmorillonite were prepared from Wyoming bentonite according to the procedure described by Shainberg et al. (1975). Kaolinite, Peerless clay No. 2, was from South Carolina.

Adsorption Measurements. The following stock solutions were prepared: TBZ, 10 ppm, and MBC, 5 ppm, dissolved in redistilled water; 0.1 *M* $CaCl_2$ and 0.1 *M* NaCl in redistilled water.

The fungicide solution was placed in a 500-ml erlenmeyer flask, $CaCl_2$ or NaCl was added, and the solution was diluted with water to give a final volume of 150 ml